



# Inactivation by solar photo-Fenton in pet bottles of wild enteric bacteria of natural well water: Absence of re-growth after one week of subsequent storage

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## ABSTRACT

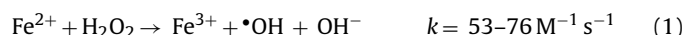
Iron photo-assisted inactivation of wild enteric bacteria (total coliforms/*E. coli* and *Salmonella* spp.) was carried out in water from the Sahelian wells having different pH (W1: 4.9 and W2: 6.3) and a natural iron content of 0.07 mg/L. We evaluate the efficiency of the disinfection on different systems containing both or only one Fenton reagent ( $\text{H}_2\text{O}_2/\text{Fe}^{2+}$ ): (i)  $\text{H}_2\text{O}_2/\text{Fe}^{2+}/h\nu$ , (ii)  $\text{Fe}^{2+}/h\nu$ , (iii)  $\text{H}_2\text{O}_2/h\nu$ , and (iv) only light irradiation ( $h\nu$ ) at lab and field scale. Generally, 0.6 mg/L of  $\text{Fe}^{2+}$  and/or 8.5 mg/L of  $\text{H}_2\text{O}_2$  were used in the Fenton reagent. The systems  $\text{H}_2\text{O}_2/\text{Fe}^{2+}/h\nu$  and  $\text{H}_2\text{O}_2/h\nu$  led to total inactivation of *Salmonella* and *E. coli*. The natural iron content (0.07 mg/L) was enough to drive an efficient photo-Fenton process leading to total bacterial inactivation. Our results show that: (i) the iron salt present in Sahelian water is enough to perform a photo-Fenton disinfection of drinking water when adding  $\text{H}_2\text{O}_2$ , (ii) addition of external iron salts at near neutral pH has no additional effect on the bacterial photo-Fenton inactivation process. After one week of storage, no enteric bacteria re-growth was observed in treated waters. Mechanistic suggestions are presented to explain the observed results.

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## 1. Introduction

The use of solar energy for water treatment is a new and promising alternative to the disinfection of drinking water. Solar disinfection (SODIS) was first used in 1980 to produce re-hydration solutions for children suffering from diarrhea in Beirut [1]. The SODIS treatment involves the use of transparent plastic bottles (Polyethylene Terephthalate (PET) of 1–1.5 L) filled with water and exposing them to sunlight for at least 6 h depending on the meteorological conditions. The inactivation or death of pathogenic microorganisms is achieved by the synergistic effect of radiation and heat [2–4]. Underexposure and bacterial re-growth result in incomplete bacterial inactivation [5,6]. Improvement of the SODIS treatment includes the use of black backs bag [7] or the  $\text{TiO}_2$  photocatalytic processes [8–10]. Recently, the photo-Fenton system ( $\text{Fe}^{2+}/\text{H}_2\text{O}_2/h\nu$ ) has been shown to increase the solar photo-inactivation of *Escherichia coli* at near-neutral pH [11–13].

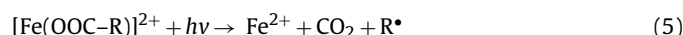
Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) significantly increased the photocatalytic inactivation process in natural water containing natural iron [14]. The Fenton reagent generates the highly reactive  $\bullet\text{OH}$  via the Haber–Weiss reaction [15]:



During the Fenton process,  $\text{Fe}^{2+}$  can be regenerated from  $\text{Fe}^{3+}$  in the presence of  $\text{H}_2\text{O}_2$ :



But the Fenton process is limited by the  $\text{Fe}^{2+}$  regeneration of  $\text{Fe}^{3+}$  (Eq. (2)). This drawback is partially countered by photo-Fenton reactions. In fact, under illumination, ferric-hydro-complexes or ferric-organo-complexes in solution can absorb photons and generate ligand-to-metal charge-transfer (LMCT) reaction in which  $\text{Fe}^{2+}$  generating  $\bullet\text{OH}$  (Eqs. (3)–(5)), [16,17].



The pH influences the efficiency of the photo-Fenton reagent, with an optimum level of pH 2.8–3 [18]. Considering this acidity criteria, the photo-Fenton was in the past preferably used for

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the treatment of wastewaters, mainly for the degradation of biorecalcitrant organic pollutants via the generated ROS [16,19]. But the recent discovery of its efficiency at near-neutral pH in the presence of organic substances [13] gives the possibility of using it in the treatment of drinking water. The Sub-Saharan African region receives about 2500–3000 h of solar radiation annually with more than 2300 KWh m<sup>-2</sup> an<sup>-1</sup> irradiance in some parts [19]. This natural illumination with a UV-component represents a powerful intake to drive photo-Fenton reactions in the region.

Studies on the inactivation of model bacteria (*E. coli* K 12) by the photo-Fenton reagent in milliQ water [11,13] or milliQ water containing simulated NOM [13] and some research on the disinfection of wild bacteria in natural water have been reported [14,20]. This study is related to the inactivation of bacteria in natural waters. In this study, we address the intervention of the Fenton (Fe<sup>2+</sup>/H<sub>2</sub>O<sub>2</sub>) reagent-driven bacterial inactivation and photo-Fenton (Fe<sup>2+</sup>/H<sub>2</sub>O<sub>2</sub>/hν) processes on various wild bacteria in groundwater from wells in Burkina Faso, West Africa.

## 2. Experimental details

### 2.1. Reagents and materials

Hydrogen peroxide, 30% (AnalaR Normapur, VWR) and Iron (II) Sulphate Heptahydrate (FeSO<sub>4</sub>·7H<sub>2</sub>O) were used to prepare the Fenton reagent. During the experiments, Catalase from bovine liver was used to inactivate the remaining H<sub>2</sub>O<sub>2</sub>, in the treated water before the bacterial culture. Hydrochloric acid fuming (HCl), 37% was used for glass-reactor cleaning. Catalase and HCl were from Fluka Analytical, SIGMA-ALDRICH®.

### 2.2. Water sampling

Water samples were collected at two household wells from two sectors in Ouagadougou: well 1 (W1) at Tanghin, sector 30, and well 2 (W2) at Nonsing, sector 21. Water from these wells is used for cooking, laundry, bathing and occasionally for drinking purposes during the recurrent water shortage period. Samples for lab experiments were collected from the water sources with two PET bottles (1.5 L) 1 h before the beginning of the process. One bottle was used to determine some physico-chemical parameters and the other for the disinfecting experiments. Water for field experiments was collected only from W2 with a 20-L plastic jerrican. In situ temperature was monitored.

### 2.3. Bacterial strain and growth media

The wild bacterial strain monitored in this study was the fecal indicator bacteria coliforms/*E. coli*, and *Salmonella* spp. Microbiology Chromocult® (Merck KGaA), was used for bacterial plating. The Chromocult is a selective and differential growth media. It selectively inhibits growth of the non-enteric bacteria. As experiments were conducted with natural water, considering their initial enteric bacteria contents, no dilution was realized before the bacterial plating. 100 µL of sample water were inoculated in the growth medium. Considering the selectivity of Chromocult, the detection limit of enteric bacteria was 0 (zero) colony growths observed in the plate. The differential nature of the medium permits the distinction of *Salmonella* spp (colorless), *E. coli* (purple and pink) and the blue and salmon colored colonies of other coliform bacteria. The incubation period was 18–24 h at 37 °C, allowing the growth of all previously mentioned enteric bacteria. However, in order to more strongly represent the decrease of the total coliforms, all the *E. coli* observed and others coliforms counted were presented together in this study.

**Table 1**

Characteristics of some physico-chemical parameters of the water sample used in field experiments.

Parameters	Experiment 1 (J1)	Experiment 2 (J2)	Experiment 3 (J3)
Turbidity (NTU)	8 ± 0.2	8 ± 0.2	9 ± 0.1
pH	6.13 ± 0.05	6.26 ± 0.02	6.14 ± 0.05
Temperature (°C)	28.9 ± 0.2	31.1 ± 0.2	29.3 ± 0.2
Initial iron content (mg/L)	0.07 ± 0.02	0.07 ± 0.02	0.07 ± 0.02
Added iron (mg/L)	0.6	0.6	0.6
Added H <sub>2</sub> O <sub>2</sub> (mg/L)	8.5	8.5	8.5

### 2.4. Analytical methods for physical parameters of water

Temperature (T °C), pH and hydrogen peroxide evolution were monitored following Sciacca et al. [14]. Turbidity was measured with a PCcompact® Turbidity/Trübung, (Aqualitic). The total iron content was measured with the spectrophotometer HACH 2000, by the FerroVer method 265. The detection limit of the spectrophotometer HACH 2000 was 0.02 ± 0.01 mg/L.

### 2.5. Helio-photo-inactivation experiments

#### 2.5.1. Laboratory experiments using a solar simulator (suntest)

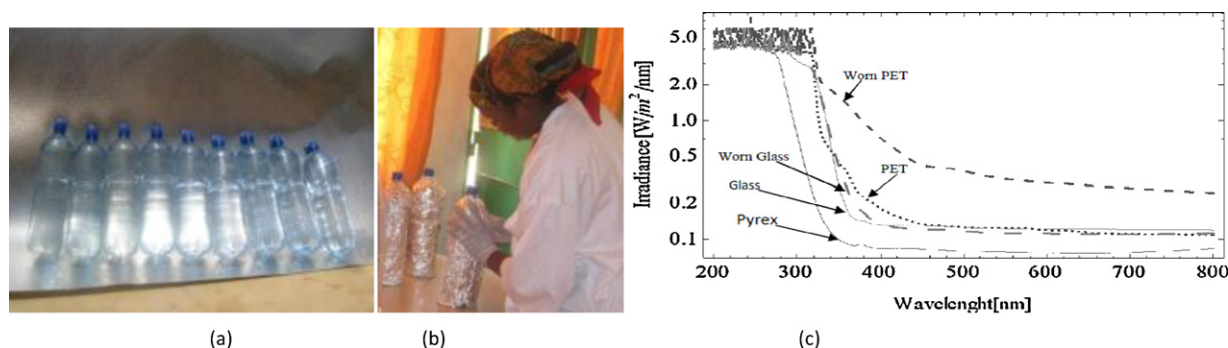
The photo-inactivation experiments at laboratory scale were conducted following the process used by Spuhler et al. [13] in a solar simulator (suntest). The disinfecting efficiency of four photo-assisted systems combined with both or only one Fenton reagent (H<sub>2</sub>O<sub>2</sub>/Fe<sup>2+</sup>) in: (i) H<sub>2</sub>O<sub>2</sub>/Fe<sup>2+</sup>/hν, (ii) Fe<sup>2+</sup>/hν, (iii) H<sub>2</sub>O<sub>2</sub>/hν, and (iv) only light irradiation (hν) were evaluated in parallel with dark-control experiments: (5) H<sub>2</sub>O<sub>2</sub>/Fe<sup>2+</sup>/obs, (6) Fe<sup>2+</sup>/obs, (7) H<sub>2</sub>O<sub>2</sub>/obs, and (8) obscurity only (obs). In these systems, hν: refers to illumination and obs: to obscurity. Glassware for analytical analysis and reactors were acid soaked after each experimental series to prevent iron cross-contamination (10% HCl, 3 days and nights). After preliminary experiments the H<sub>2</sub>O<sub>2</sub> dose in this study was set at 8.5 mg/L. The concentration of the added iron was Fe<sup>2+</sup> (0.6 mg/L) as evaluated by Spuhler et al. [13]. The initial pH of the water was 4.9 and 6.3 respectively for W1 and W2. Each experiment was repeated at least three times to ensure the reproducibility of the results.

#### 2.5.2. Field experiment using PET bottles

PET bottles were used for field experiments as they have a relative good absorbance (Fig. 1c). Only the water from W2 with a close-to-neutral pH (Table 1) was used at this level. Following the results of the lab experiments, the system Fe<sup>2+</sup>/hν was not evaluated at field scale and the other systems and their blank were evaluated in triplicate (Fig. 1a and b) over three successive days at the end of April 2010. Nine new PET bottles (1.5 L) representing three replications of the systems (H<sub>2</sub>O<sub>2</sub>/Fe<sup>2+</sup>/hν, H<sub>2</sub>O<sub>2</sub>/hν, and (hν)) were filled with: Fe<sup>2+</sup> (0.6 mg/L) and H<sub>2</sub>O<sub>2</sub> (8.5 mg/L) added before their exposure to solar radiation. Blank control bottles were covered with Al-foil and kept in the dark. During the experiments, solar radiation was recorded following the process used by Sciacca et al. [20]. To evaluate the bacterial inactivation rate, samples of the treated water were collected at regular time intervals in a sterile 15 mL Eppendorf for plating. Some parameters of these water samples are presented in Table 1. The PET bottles were used only once to ensure the same and relatively good light transmittance (Fig. 1c) in all the experiments.

### 2.6. Post-irradiation events

At the end of the irradiation phase of the laboratory experiments, remaining samples from systems 1 and 2 were introduced into sterile 50 mL Eppendorf flasks and kept in the dark at room



**Fig. 1.** (a) 9 PET bottles for the triplicate simultaneous exposure of the systems  $\text{H}_2\text{O}_2/\text{Fe}^{2+}/h\nu$ ,  $\text{H}_2\text{O}_2/h\nu$ , and  $h\nu$  (irradiation only). (b) Blank of each system, (c) Absorbance of different reactor materials (Pyrex, Glass, PET).

temperature varying from 25 to 30 °C. For field experiments, each PET bottle from the exposed and blank tests was closed and kept in the dark. Re-growth experiments were realized on stored bottles after 24 h, 72 h and one week. Considering the real scale situation for treated water intended for populations, the water samples were kept in the dark without removing their remaining 2–3 mg/L of  $\text{H}_2\text{O}_2$ . This remaining amount of  $\text{H}_2\text{O}_2$  ensures a residual effect on the treatment. However, it was no more detectable after 48–72 h.

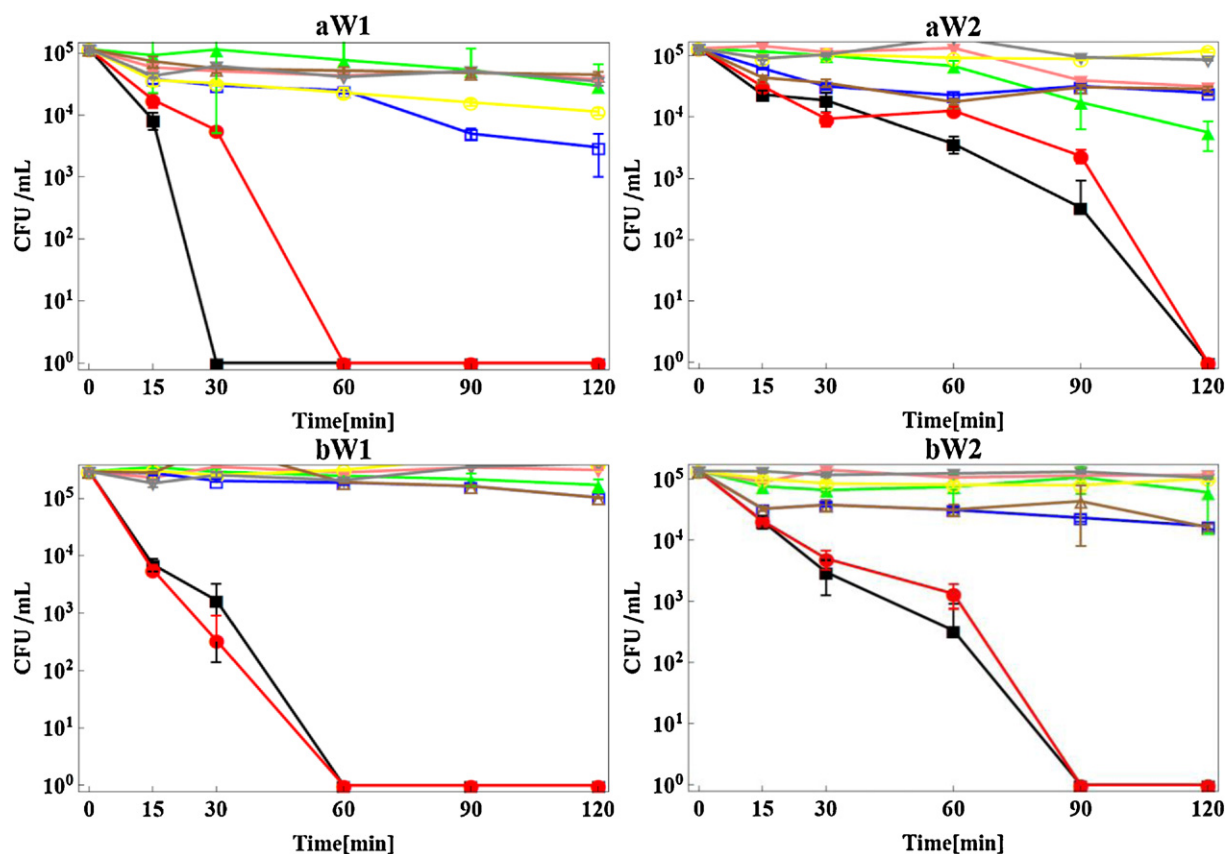
### 2.7. Data analysis

The three-way ANOVA Package of the Wolfram Mathematica 8.0 program was used to evaluate the influence of the acidity, bacteria types and irradiation used on the disinfection rate.

## 3. Results

### 3.1. Physico-chemical characterization

Both water samples used in this study were collected at Ouagadougou during the months of March and April 2010 (dry season). They had an initial temperature of 29 °C and low turbidity < 10 NTU. The maximum acceptable turbidity recommended for SODIS disinfection is 30 NTU, [4,6]. The W1 has an initial pH of 4.9 and a total iron content of 0.06 mg/L; while in W2 the initial pH value was 6.3 and the total iron content 0.07 mg/L. The concentration of both wild enteric bacteria (total coliforms/*E. coli* and *Salmonella* spp.) was approximately  $10^5$  CFU/mL in both water sources.



**Fig. 2.** Inactivation of the bacteria content in sample water from well 1 (W1, pH:  $4.9 \pm 0.05$ ) and well 2 (W2, pH:  $6.3 \pm 0.05$ ) during photocatalytic treatment in the solar simulator Suntest. After the introduction of 90 ml of sample water to the 100 ml glass reactor, 8.5 mg/L of  $\text{H}_2\text{O}_2$  and 0.6 mg/L of  $\text{Fe}^{2+}$  were added to the corresponding systems and their dark control. During the experiments, water temperature was < 45 °C. (a) Total coliforms/*E. coli*, (b) *Salmonella* spp. (■)  $\text{Fe}^{2+}/\text{H}_2\text{O}_2/h\nu$ , (●)  $\text{H}_2\text{O}_2/h\nu$ , (▲)  $\text{Fe}^{2+}/h\nu$ , (▼)  $h\nu$  only, (□)  $\text{Fe}^{2+}/\text{H}_2\text{O}_2/\text{Obs}$ , (○)  $\text{H}_2\text{O}_2/\text{Obs}$ , (△)  $\text{Fe}^{2+}/\text{Obs}$  and (▽)  $\text{Obs}$  only. Graphics produced by the ListLogPlot function of Wolfram Mathematica software.

### 3.2. Experiment under simulated solar radiation

As it has been reported before [13,21], the decrease in CFU/mL of the samples' enteric bacteria broadly follows the first order kinetics, based on log-linear plots (Fig. 2). Inactivation rate constants observed during the inactivation process reported as  $k$  [ $\text{min}^{-1}$ ] were calculated by linear regression (Table 2). Considering all the systems tested with W1 and W2, only the photo-Fenton ( $\text{Fe}^{2+}/\text{H}_2\text{O}_2/h\nu$ ) and hydrogen peroxide systems ( $\text{H}_2\text{O}_2/h\nu$ ) lead to a total inactivation of the fecal indicators bacteria. Irradiation only ( $h\nu$ ) and  $\text{Fe}^{2+}/h\nu$ , as well as blank systems ( $\text{H}_2\text{O}_2/\text{Fe}^{2+}/\text{obs}$ ,  $\text{Fe}^{2+}/\text{obs}$ ,  $\text{H}_2\text{O}_2/\text{obs}$ ,  $\text{obs}$ ) show a lower decrease in their active enteric bacteria concentration within 2 h' irradiation (Fig. 2).

#### 3.2.1. Inactivation in the systems $\text{Fe}^{2+}/\text{H}_2\text{O}_2/h\nu$ and $\text{H}_2\text{O}_2/h\nu$

Considering only the systems in which the total inactivation of the bacteria were achieved ( $\text{Fe}^{2+}/\text{H}_2\text{O}_2/h\nu$  and  $\text{H}_2\text{O}_2/h\nu$ ), it can be noticed that in W1 (pH=4.9) the photo-Fenton system ( $\text{Fe}^{2+}/\text{H}_2\text{O}_2/h\nu$ ) induced a stronger inactivation of the enteric bacteria, particularly in the total coliforms/*E. coli* group. This group has shown an inactivation rate constant of ( $k = -0.3887 \pm 0.005 \text{ min}^{-1}$ ), (Table 2) and their total inactivation was achieved after 30 min. In the  $\text{H}_2\text{O}_2/h\nu$  system, we have to take into account the natural presence of 0.07 mg/L of iron in water. Natural iron confers to this system the photocatalytic properties, leading to a high inactivation rate constant ( $k = -0.1953 \pm 0.003 \text{ min}^{-1}$ ). Total inactivation was achieved in about 60 min. In both systems, the *Salmonella* spp. concentration was totally inactivated after about 60 min.

The ranking of the two photocatalytic systems considering the inactivation rate constant (Table 2), gives the following for both wild enteric bacteria at pH 4.9 (W1):

$$k_{\text{C}}^{\text{Fe}^{2+}/\text{H}_2\text{O}_2/h\nu} > k_{\text{S}}^{\text{H}_2\text{O}_2/h\nu} > k_{\text{S}}^{\text{Fe}^{2+}/\text{H}_2\text{O}_2/h\nu} > k_{\text{C}}^{\text{H}_2\text{O}_2/h\nu}$$

At pH 6.3 (W2), the ranking was as follows:

$$k_{\text{S}}^{\text{Fe}^{2+}/\text{H}_2\text{O}_2/h\nu} > k_{\text{S}}^{\text{H}_2\text{O}_2/h\nu} > k_{\text{C}}^{\text{Fe}^{2+}/\text{H}_2\text{O}_2/h\nu} > k_{\text{C}}^{\text{H}_2\text{O}_2/h\nu}$$

C stands for total coliforms/*E. coli* and S for *Salmonella* spp.

The *Salmonella* spp. total inactivation was achieved before the total coliforms/*E. coli* group in both systems in approximately 90 and 120 min respectively.

#### 3.2.2. Inactivation in the systems $h\nu$ and in all the blank systems ( $\text{H}_2\text{O}_2/\text{Fe}^{2+}/\text{obs}$ , $\text{Fe}^{2+}/\text{obs}$ , $\text{H}_2\text{O}_2/\text{obs}$ , $\text{obs}$ )

For water from W1 (pH: 4.9), the irradiation ( $h\nu$ ) alone and all the blank systems, gives just a slight inactivation of the total coliforms/*E. coli* after the 120 min of exposure. The *Salmonella* spp. content of the sample show an increase instead of a decrease at the end of the period of irradiation (120 min), with an increasing rate constant of ( $k = 0.0013 \pm 0.005 \text{ min}^{-1}$ ). Its concentration increased also in some blank tests carried out in the dark (Table 2). A slight decrease was observed both in the Fenton system  $\text{Fe}^{2+}/\text{H}_2\text{O}_2/\text{obs}$  and the system  $\text{Fe}^{2+}/\text{obs}$  with the same inactivation rate constant ( $k = -0.0080 \pm 0.006 \text{ min}^{-1}$ ).

The inactivation of the total coliforms/*E. coli* under  $h\nu$  alone and in the W2 (pH: 6.3) blanks show a slight decrease as in W1. The *Salmonella* spp. content in the sample showed a slight increase in the system  $h\nu$  ( $k = 0.0001 \pm 0.005 \text{ min}^{-1}$ ). All the blank systems give rise to a slight decrease.

#### 3.2.3. Inactivation in the systems $\text{Fe}^{2+}/h\nu$

The system  $\text{Fe}^{2+}/h\nu$  did not lead to a total inactivation of enteric bacteria in both waters (W1 and W2). But a slight inactivation was observed, as presented in Fig. 2 and by the inactivation rate constants (Table 2).

**Table 2**  
Inactivation rate constants  $k$  [ $\text{min}^{-1}$ ] of each enteric bacteria group observed during the inactivation process, calculated by linear regression for the different photo-catalytic treatments and their corresponding blank conducted in the dark.

Water origin/pH	Enteric bacteria	Treatments/ $k$ [ $\text{min}^{-1}$ ]					
		$\text{Fe}^{2+}/\text{H}_2\text{O}_2/h\nu$	$\text{H}_2\text{O}_2/h\nu$	$\text{Fe}^{2+}/h\nu$	$h\nu$	$\text{Fe}^{2+}/\text{H}_2\text{O}_2/\text{Obs}$	$\text{H}_2\text{O}_2/\text{Obs}$
Wells 1 pH: 4.9	Total coliforms/ <i>E. coli</i>	$-0.3887 \pm 0.005$	$-0.1953 \pm 0.003$	$-0.0124 \pm 0.005$	$-0.0055 \pm 0.008$	$-0.0284 \pm 0.011$	$-0.0161 \pm 0.005$
	<i>Salmonella</i> spp.	$-0.2085 \pm 0.002$	$-0.2110 \pm 0.005$	$-0.0044 \pm 0.012$	$0.0013 \pm 0.005$	$-0.0080 \pm 0.006$	$0.0041 \pm 0.006$
Wells 2 pH: 6.3	Total coliforms/ <i>E. coli</i>	$-0.1001 \pm 0.011$	$-0.0798 \pm 0.007$	$-0.0260 \pm 0.011$	$-0.0133 \pm 0.004$	$-0.0105 \pm 0.015$	$-0.0002 \pm 0.003$
	<i>Salmonella</i> spp.	$-0.1380 \pm 0.004$	$-0.1189 \pm 0.006$	$-0.0025 \pm 0.003$	$0.0001 \pm 0.005$	$-0.0125 \pm 0.002$	$-0.0016 \pm 0.002$
						$\text{Fe}^{2+}/\text{Obs}$	$\text{Obs}$
						$-0.0062 \pm 0.002$	$-0.0063 \pm 0.004$
						$-0.0080 \pm 0.006$	$0.0045 \pm 0.003$
						$-0.0103 \pm 0.007$	$-0.0024 \pm 0.003$
						$-0.0125 \pm 0.002$	$-0.0013 \pm 0.005$



**Table 3**  
Three-way ANOVA analysis results.

Parameters	DF	SS	MS	F	P
pH	1	21,930.8	21,930.8	48.66	0.001%
Bacteria species	1	1201.25	1201.25	2.65	11%
Treatments	1	826.875	826.88	1.83	18%
pH/bacteria species	1	396.75	396.75	0.88	35%
pH/treatments	1	6.13	6.13	0.01	91%
Bacteria species/treatments	1	585.21	585.21	1.30	26%
Error	73	32,901.8	450.71	–	–
Total	79	57,848.8	–	–	–

DF: degree of freedom; SS: sum of square; MS: means square; F: Fisher factor (influence factor); P: probability.

The data shown in Fig. 2 and Table 2, for a single day experiment, present experiments carried out in triplicate. Experiments were repeated on three different days and a similar inactivation rate was observed.

### 3.2.4. Influence of pH, bacteria species and inactivation system on the disinfection

A significant difference between the inactivation rate of the total enteric bacteria concentration of both wells (W1: pH 4.9 and W2: pH 6.3) was observed when applying  $\text{Fe}^{2+}/\text{H}_2\text{O}_2/h\nu$  and  $\text{H}_2\text{O}_2/h\nu$ . The analysis of these processes by the three-way ANOVA program of Mathematica 8.0, allowed us to evaluate the influence of the acidity and other parameters, as presented in Table 3. With a Fisher ratio or influence factor ( $F$ ) of about 48.66, it is possible to state that the pH has a strong impact on the photo-catalytic disinfection process [16]. The probability ( $P=0.001\%$ ) gives rise to the assumption that the error may be due to noise. The impact of bacteria (total coliforms/*E. coli* or *Salmonella* spp.) or the treatment ( $\text{Fe}^{2+}/\text{H}_2\text{O}_2/h\nu$  or  $\text{H}_2\text{O}_2/h\nu$ ) on the disinfection process was less significant. The probability that the difference in the inactivation rate related to these two parameters could be due to experimental error was about 11% and 18% respectively for the bacteria species and treatment used. The cross-interactions between the three parameters did not significantly affect the data obtained.

### 3.2.5. Post-irradiation effect

To be sure that the inactivated bacteria was not just partly damaged, rather than killed, when using the photo-Fenton ( $\text{Fe}^{2+}/\text{H}_2\text{O}_2/h\nu$ ) or hydrogen peroxide ( $\text{H}_2\text{O}_2/h\nu$ ) systems under light irradiation, after completion of the runs the samples were transferred into sterile flasks and kept at 25–30 °C in the dark (*obs*). Further spread plate counts were performed on the samples after 24 h, 72 h and up to one week. No re-growth of coliforms/*E. coli* or *Salmonella* spp. was observed, and this suggested irreversible inactivation.

## 3.3. Field experiments

Field experiments indicated that the inactivation rate of both bacteria types applying  $\text{Fe}^{2+}/\text{H}_2\text{O}_2/h\nu$  and  $\text{H}_2\text{O}_2/h\nu$  was not significantly affected by the difference observed in the solar radiation and temperature each day. Variation in the inactivation rates were observed, however, when light alone was applied. As presented in all the graphs in Fig. 3, any variation of the solar radiation influences the inactivation of the bacteria. Some of the coliforms/*E. coli* inactivation curves have a shoulder. However, the decrease in CFU mL<sup>−1</sup> of most of the enteric bacteria curves follows first-order kinetics, as in the lab experiments, based on log-linear plots.

### 3.3.1. Inactivation under direct solar radiation only (*hv*)

**3.3.1.1. Inactivation total coliforms/*E. coli*.** The temperature of 49 °C and UV irradiation of more than 30 W/m<sup>−2</sup> during the first exposure gave rise to the total inactivation of coliforms/*E. coli* within 90 min. On the second day, irradiation was reduced and decreased from 26 W/m<sup>−2</sup> to 20 W/m<sup>−2</sup> during the first exposure. Consequently, inactivation was achieved after 120 min only. No re-growth was observed after 24 h of storage in the dark over these two days. On the third day, the reduction in temperature to less than 45 °C and high fluctuation in the solar radiation (between 23 and 14 W/m<sup>−2</sup>) did not permit a total inactivation of coliforms/*E. coli* in this system (*hv*). Inactivation of the blank (*obs*) kept in the dark with a water temperature of around 38 °C was not significant during the time of experimentation (2 h). But after the 24 h dark-storage period, inactivation of about 97% was observed on the first and third day, and total inactivation on the second.

**3.3.1.2. Inactivation of *Salmonella* spp..** The favorable conditions during the first day of experimentation enhanced considerably the inactivation of *Salmonella* spp.: about 96% of the population was inactivated during the 2 h of exposure. On the second day, the relatively low irradiation gave rise to a total inactivation at the end of the illuminated process, but a recovery of the viability during the 24 h dark-storage period was observed (Fig. 3b2). The atmospheric conditions on the third day were not favorable and only 44% was inactivated at the end of the exposure. Blank tests in the system *obs*, did not give a significant inactivation during the experiments and after the 24 h of dark storage, their concentration remained constant.

### 3.3.2. Inactivation under enhanced systems $\text{Fe}^{2+}/\text{H}_2\text{O}_2/h\nu$ and $\text{H}_2\text{O}_2/h\nu$

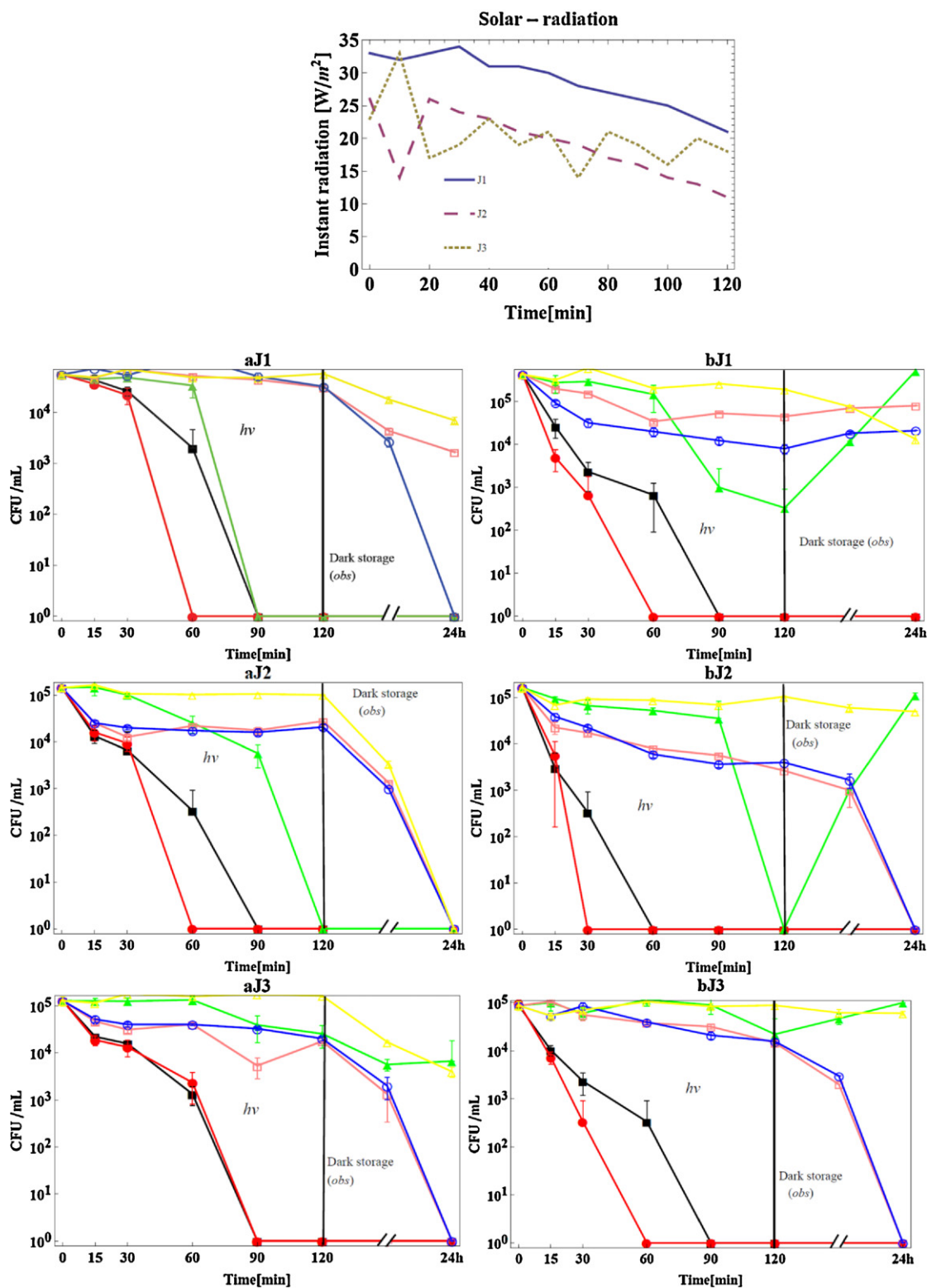
$\text{Fe}^{2+}/\text{H}_2\text{O}_2/h\nu$  and  $\text{H}_2\text{O}_2/h\nu$  showed a significant increase in the inactivation rate of bacteria compared to the non-enhanced systems (*hv*) (Fig. 3). Moreover, the variation of the solar radiation over the three days did not significantly influence the inactivation kinetics. For both bacteria species,  $\text{H}_2\text{O}_2/h\nu$  showed a higher inactivation rate than that of the systems with added iron ( $\text{Fe}^{2+}/\text{H}_2\text{O}_2/h\nu$ ). The added iron has precipitated in the system at pH > 6. An exception was observed in the case of total coliforms/*E. coli* on the third day (Fig. 3aJ3), because they were totally inactivated in both systems. This could be related to the variability of the daily solar radiation. Re-growth experiments for both systems during 24 h did not show any bacterial recovery.

For total coliforms/*E. coli*, the control systems ( $\text{Fe}^{2+}/\text{H}_2\text{O}_2/obs$  and  $\text{H}_2\text{O}_2/obs$ ) did not show significant inactivation during the experiments. However, the inactivation occurred after the 24 h of dark storage for the second and third day. It's occurred only in the system  $\text{H}_2\text{O}_2/obs$  the first day. As in the previous cases, the control systems did not lead to a significant inactivation of *Salmonella* spp. during the experiment. But after the dark storage period (24 h), total inactivation was observed in both systems on the second and third day. None of the systems led to total inactivation on the first day.

## 4. Discussions

### 4.1. Irradiation characteristics

The efficiency of the solar photocatalytic disinfection of water can be influenced by the sun's intensity, light absorption, initial bacterial concentration, water temperature and turbidity [12–14,22]. In the lab experiments, the photo inactivation was carried out under continuous irradiation at constant intensity, with a radiation intensity of 560 W m<sup>−2</sup>. This corresponds to 300–400 nm UVA or approximately 32 W m<sup>−2</sup> UVA, representing the average UVA radiation of Ouagadougou in summer [19]. During the lab experiments,



**Fig. 3.** Inactivation of the bacteria contained in water sample from wells 2 (W2, pH: 6.3) during the field experiment under direct solar radiation. After the introduction of 1.5 L of water in to the 1.5 L PET reactor, 8.5 mg/L of  $\text{H}_2\text{O}_2$  and 0.6 mg/L of  $\text{Fe}^{2+}$  were added to the corresponding systems and their dark control. (a) Total coliforms/*E. coli*, (b) *Salmonella* spp., (J1) 28/04/2010, (J2) 29/04/2010, (J3) 30/04/2010, (■)  $\text{Fe}^{2+}/\text{H}_2\text{O}_2/h\nu$ , (●)  $\text{H}_2\text{O}_2/h\nu$ , (▲)  $h\nu$  only, (□)  $\text{Fe}^{2+}/\text{H}_2\text{O}_2/\text{Obs}$ , (○)  $\text{H}_2\text{O}_2/\text{Obs}$ , (△)  $\text{Obs}$  only. Graphs produced by the ListLogPlot function of Wolfram Mathematica software.

the water temperature inside the batch reactor remained inferior to 45 °C and thermal inactivation can be excluded [2,3]. Direct DNA damage by UVB can also be excluded as the used solar simulator emits negligible amounts of photons at wavelengths shorter than

300 nm [8] and the reactor material screened UVB (280–320 nm, Fig. 1c). It has to be noted that the water matrix used in this study is natural water, contrary to the laboratory milliQ-water which contains NOM and exogenous photosensitizers.

## 4.2. Experiments under solar simulator

### 4.2.1. Iron system ( $\text{Fe}^{2+}/h\nu$ )

In solutions at low pH (2–3), the irradiation with UV of various hydroxylated  $\text{Fe}^{3+}$  species produces  $\text{Fe}^{2+}$  and the hydroxyl radical  $\text{OH}^\bullet$  (Eqs. (3) and (4) [17,18]. The generated  $\text{OH}^\bullet$  radicals are highly oxidant, as explained above. But in natural water (pH: 4.9 and 6.3 respectively), the photoactive ferric hydrolyzed molecules  $[\text{Fe}(\text{OH})^{2+}]$  are not soluble, as the predominant iron component of this pH is the iron-complex which under irradiation generates  $\text{Fe}^{2+}$ , with an organic radical instead of  $\text{OH}^\bullet$  (Eq. (5)) [16]. During the photocatalytic inactivation process in the  $\text{Fe}^{2+}/h\nu$  system, the bactericidal effect of  $\text{Fe}^{2+}$  arises from its ability to diffuse into the cells, leading to the generation of  $\text{OH}^\bullet$  via intracellular Fenton reactions when reacting with metabolic  $\text{H}_2\text{O}_2$  [23,24]. Spuhler et al. [13], observed a lethal action of the system  $\text{Fe}^{2+}/h\nu$  during the inactivation of *E. coli* K12, in MilliQ Water ( $h\nu > 290$  nm, pH: 5–5.5) resulting in a total inactivation after 120 min. However, in the present study neither the wild total coliforms/*E. coli* nor the *Salmonella* spp. were totally inactivated after the same exposure period in well water (W1, pH 4.9; W2: pH 6.3). The difference in this and Spuhler et al. [13] results could be explained by the nature of the water and bacteria species and by the following pathway: (i) The inactivation process through the ROS generated after the excitation of the exogenous and endogenous photosensitizers was not sufficient to ensure the total inactivation of the wild enteric bacteria involved; (ii) after the active ROS production (20–30 min), injured bacteria have developed self-repair mechanisms [11] and became more resistant to the light irradiation, and multiplied (iii) the wild bacteria are more resistant than the manufactured *E. coli* strain regularly used in lab experiments; (iv) the natural water matrix used here contains NOM and other minerals substances. These bacteria cannot create an osmotic stress as in MilliQ water which could weaken the bacteria and support the introduction of  $\text{Fe}^{2+}$  into the bacteria leading to intra-cellular Fenton ROS inactivation.

### 4.2.2. Systems $\text{Fe}^{2+}/\text{H}_2\text{O}_2/h\nu$ and $\text{H}_2\text{O}_2/h\nu$

Natural water in the Sahelian region contains large quantities of iron as it flows on ferruginous substrates [14,20,25]. It is introduced into the atmosphere by wind and is found in aerosols, fog, rain drops, ground water and lakes [18]. A total initial iron concentration of about 0.06 mg/L and 0.07 mg/L was detected in W1 and W2 respectively. The high inactivation rate observed in the system  $\text{H}_2\text{O}_2/h\nu$  for both wells as in the systems  $\text{Fe}^{2+}/\text{H}_2\text{O}_2/h\nu$  in contrast to that of the systems  $\text{Fe}^{2+}/h\nu$  or  $h\nu$  only can allow us to assume that the photo-Fenton also takes place in the system  $\text{H}_2\text{O}_2/h\nu$  by using the initial iron contained as the catalyst. From Fig. 2 and Table 2, the difference in the iron content for the systems  $\text{Fe}^{2+}/\text{H}_2\text{O}_2/h\nu$  (natural iron + added iron) and  $\text{H}_2\text{O}_2/h\nu$  (natural iron only) did not significantly influence the inactivation kinetic of both bacteria species in both wells. It can be underlined that it is only in the case of total coliforms/*E. coli* in water from W1 that the difference between the inactivation rates was significant for different photo-catalytic systems. In the remaining systems (*Salmonella* spp. of W1 and total coliforms/*E. coli* and *Salmonella* spp. of W2), the total inactivation was achieved approximately within similar times for both photocatalytic systems and no significant differences were observed in their inactivation rate constants ( $k$ ). Considering the initial iron content of water from the Sahelian region, it seems possible to achieve disinfection of water by photo-Fenton process without adding extra iron. This would be a great contribution, not only in reducing the chemical inputs required for the application of the photo-Fenton system for the treatment of drinking water, but also in reducing treatment costs.

### 4.2.3. Illumination alone ( $h\nu$ )

The total inactivation of total coliforms/*E. coli* or *Salmonella* spp. was not observed in the reactor under the effect of light irradiation alone. This could be related to the fact that the exposure time was just 2 h, and not 5 or 6 h as recommended for the SODIS process [6]. As the present study was focused on the reduction of the solar disinfection exposure time by the photo-Fenton, the 2 h exposure were sufficient to obtain a significant inactivation rate using  $\text{Fe}^{2+}/\text{H}_2\text{O}_2/h\nu$  and  $\text{H}_2\text{O}_2/h\nu$ .

### 4.2.4. pH

Great differences and contradictions were observed in the W2 (pH 6.3) in both systems where total inactivation was achieved, as the *salmonella* spp. strain was the first to be totally inactivated in both systems in about 90 min, while that of total coliforms/*E. coli* took about 120 min (Fig. 2). This situation is in contrast with the assumption that *Salmonella* spp. is more resistant to photocatalytic inactivation than *E. coli* [14,26]. Indeed, even in the W1 (pH: 4.9), in the system  $\text{H}_2\text{O}_2/h\nu$ , both enteric bacteria species were inactivated approximately at the same time and the inactivation rates constant of *Salmonella* spp. were slightly greater than those of total coliforms/*E. coli* ( $-0.2110 \pm 0.005 > -0.1953 \pm 0.003$ ) (Table 2). It is only in the system  $\text{Fe}^{2+}/\text{H}_2\text{O}_2/h\nu$  that the *E. coli* was rapidly inactivated before the *Salmonella* spp.

### 4.2.5. Control experiments: ( $\text{H}_2\text{O}_2/\text{Fe}^{2+}/\text{obs}$ , $\text{Fe}^{2+}/\text{obs}$ , $\text{H}_2\text{O}_2/\text{obs}$ , $\text{obs}$ )

None of the control systems (blank) led to total inactivation of the wild enteric bacterial concentration, even though a slight inactivation was observed in some cases for both wells. These results correspond with previous studies [11,13]. However, it was noticed that total coliforms/*E. coli* inactivation was more pronounced in W1 (pH: 4.9) than in W2 (pH: 6.3) (Table 2), but that situation was the reverse for the *Salmonella* spp. inactivation, whose population even increased in some systems ( $\text{H}_2\text{O}_2/\text{obs}$  and  $\text{obs}$ ).

## 4.3. Experiments under direct solar radiation

The decrease of the enteric bacterial amount and even its total inactivation in the control systems ( $\text{Fe}^{2+}/\text{H}_2\text{O}_2/\text{obs}$  and  $\text{H}_2\text{O}_2/\text{obs}$ ), after the 24 h of dark storage could be due to the scavenging action of  $\text{H}_2\text{O}_2$  as it is also a powerful ROS [13,22]. It should be noticed that at high pH (6.3) in the dark, the iron precipitation makes them unavailable to initiate the simple Fenton reaction (Eq. (1)) [16]. The  $h\nu$  and  $\text{obs}$  systems are expected not to produce ROS during the 24 h dark storage, thus explaining why no more inactivation was observed in the remaining total coliforms/*E. coli* and for *Salmonella* spp. The increased concentration after the storage, could be due to the fact that during the dark storage without ROS production to injure them, the bacteria recover their ability to grow and replicate [11]. For total coliforms/*E. coli*, no re-growth was observed after total inactivation. The re-growth of *Salmonella* spp. was due to resistance to solar disinfection (not enhanced), as recently reported [26].

For the enhanced systems,  $\text{H}_2\text{O}_2/h\nu$  shows a better inactivation kinetic than that of the systems containing added iron ( $\text{Fe}^{2+}/\text{H}_2\text{O}_2/h\nu$ ). It can be assumed that with the low Fe-concentration in natural water (0.07 mg/L), this amount was enough to start, in the presence of  $\text{H}_2\text{O}_2/h\nu$ , the photo-Fenton process and generate  $\text{OH}^\bullet$  inactivating of the enteric bacteria. The higher inactivation kinetic of the systems  $\text{H}_2\text{O}_2/h\nu$  compared to  $\text{Fe}^{2+}/\text{H}_2\text{O}_2/h\nu$  may suggest that a high iron concentration could have a negative effect on the photo-Fenton treatment of natural water close to neutral pH. This negative effect could be due to: (i) iron precipitation resulting in the lack of soluble iron in the medium to maintain the photocatalytic cycle, (ii) the reduction of

light transmittance due the increased turbidity of water and its coloration due to high iron contents, (iii) excess iron concentration in the solution increasing the  $\text{OH}^\bullet$  scavenging potential (Eq. (6)) and concomitantly reduce the efficiency of the process [17].



On the third day, the *Salmonella* spp. showed approximately the same inactivation kinetic as that of the first day under the best atmospheric conditions. Inactivation on the second day, under the same temperature but lower irradiation ( $26\text{--}20\text{ W m}^{-2}$ ) was higher than on the first and third day. These observations are related to the results for total coliforms/*E. coli*, which show also approximately the same inactivation kinetic on the first and second day. It could be assumed that up to a certain level of irradiation and temperature, the influence of the two parameters (temperature and irradiation intensity) on the photo-Fenton inactivation process is no longer significant. The same observation was made previously by Ubomba-Jaswa et al. [27] during the investigations into the effect of the UVA dose on the inactivation of *E. coli* K12. However, a slight reduction of the inactivation kinetic of total coliforms/*E. coli* on the third day in the systems  $\text{H}_2\text{O}_2/\text{h}\nu$  [11] is indicative that intermittent irradiation associated with low irradiation has negative influence on the bacterial inactivation rate.

#### 4.4. Inactivation pathways

##### 4.4.1. Inactivation in the illuminated system

In all the illuminated systems, part of the observed photo-inactivation could be due to excitation of exogenous (ferric-hydro-complex or ferric-organo-complex) and endogenous (cytochrome, flavin, tryptophan) photosensitizers, [16,17] as well as the ROS-action ( $^1\text{O}_2$ ,  $\text{O}_2^{\bullet-}$ ,  $\text{OH}^\bullet$  and  $\text{H}_2\text{O}_2$ ) generated from the dissolved oxygen ( $\text{O}_2$ ) contained naturally in the water via successive steps of one-electron reductions [17,23]. The  $\text{O}_2^{\bullet-}$  and/or the  $\text{H}_2\text{O}_2$  have the ability to attack proteins and cell membrane components, especially membrane lipids, resulting in their peroxidation [23]. This peroxidation increases the cell membrane permeability and the disruption of the trans-membrane ion gradients [6], which can lead to the inactivation of the cells.  $\text{H}_2\text{O}_2$  is a non-charged molecule and penetrates readily the cellular membranes [13]. The toxicity of  $\text{H}_2\text{O}_2$  is due to the fact that they can induce the production of  $\text{OH}^\bullet$  through the Fenton reaction (Eq. (1)) within the cell [23].  $\text{OH}^\bullet$  is a highly reactive oxidant, which can degrade non-biodegradable chemical components [16,19], NOM [12,13] and inactivate bacteria [13,14].

##### 4.4.2. Inactivation of defense mechanisms

The deficiency in the cellular defense against ROS, constituted by enzymes such as SOD/SOR which control the  $\text{O}_2^{\bullet-}$  or catalase which regulate the  $\text{H}_2\text{O}_2$  concentration [23], can result in an oxidative stress leading to the increase of the ROS content of the cells at the level exceeding their defense capacity [24,28]. This deficiency in self defense mechanisms can arise from the exposure of these enzymes to thermal or optical inactivation [2]. Ghadermarzi and Moosavi-Movahedi [29] suggested that inactivation arises when the temperature is around  $45^\circ\text{C}$ . Considering the temperature measured during the photo-inactivation process in this study, it can be assumed that the enzymes for self-defense mechanisms were not efficient, giving rise to the optical inactivation through UVA radiation to efficiently inactivate the bacterial contents of the water [3]. The inactivation of the SOD/SOR and catalase leads to the increase of intracellular ROS with the direct attack of membrane and other proteins. Followed by the generation of the highly reactive  $\text{OH}^\bullet$  via intracellular Fenton reaction (Eq. (1)). This reaction take place between the  $\text{H}_2\text{O}_2$  and the iron liberated from the iron sulfur clusters ( $[\text{4Fe-4S}]$ ) after the inactivation of clusters enzymes

like dihydroxy-acid deshydratase, aconitase B and fumarases A and B [24] by the  $\text{O}_2^{\bullet-}$  [30]. The liberation of free iron in the cell comes from the specific oxidizing action of the superoxide on the centers  $[\text{4Fe-4S}]$  of these hydrolytic enzymes [24,30]. The  $\text{OH}^\bullet$  attacks lead to important cellular damage on DNA [24]. When the bacteria are not sufficiently exposed to illumination, they can recover viability by self-defense mechanisms in a short time [8]. In this study, the systems  $\text{Fe}^{2+}/\text{h}\nu$  or  $\text{h}\nu$  have showed a slight initial inactivation in both wells in about 20 or 30 min depending on the enteric bacteria involved (Fig. 2). After this time, their concentration in the water have increased and stabilized till the end of the 2 h of irradiation. This situation could be explained by the recovery of the self-defense mechanisms suggested by Rincon and Pulgarin [8]. After such recovery, the actors of the defense constituted by SOD and catalase, which have been certainly weakened but not inactivated, have recovered their properties and eliminated the exceeding ROS ( $\text{O}_2^{\bullet-}$ ,  $\text{H}_2\text{O}_2$ ), giving rise to a recovery in the damaged bacteria, which multiplied and stabilized in the medium, (see Fig. 2). In the systems,  $\text{Fe}^{2+}/\text{H}_2\text{O}_2/\text{h}\nu$  and  $\text{H}_2\text{O}_2/\text{h}\nu$ , the photo-Fenton under light and high or low iron content increase the  $\text{OH}^\bullet$  production. This increase led to an increased inactivation of wild enteric bacteria at high inactivation rates compared to those found for  $\text{Fe}^{2+}/\text{h}\nu$  or  $\text{h}\nu$ . The  $\text{Fe}^{3+}$ -bacteria interaction are enhanced in the presence of  $\text{H}_2\text{O}_2$  with the increased production of  $\text{OH}^\bullet$  and fast  $\text{Fe}^{2+}/\text{Fe}^{3+}$  interconversion under illumination [13].  $\text{OH}^\bullet$  is the most powerful oxidant generated inside the cells. It reacts instantly with no selectivity, at the diffusion limits, with sugars, amino acids, phospholipids, nucleotides and organic acids including DNA [31]. Cellular defense mechanisms against a DNA attack by  $\text{OH}^\bullet$  do not to exist.

## 5. Conclusions

This study showed that the  $\text{H}_2\text{O}_2/\text{h}\nu$  was as efficient as the photo-Fenton system ( $\text{Fe}/\text{H}_2\text{O}_2/\text{h}\nu$ ) in significantly increasing the inactivation rate of the enteric bacteria contents of the water wells. The iron naturally present in the well water influences the reaction mechanism of  $\text{H}_2\text{O}_2/\text{h}\nu$  by favoring the photo-Fenton process. The efficiency of the system  $\text{H}_2\text{O}_2/\text{h}\nu$ /natural water in lab experiments under simulated solar radiation was confirmed in the field in PET bottles showing a better inactivation rate than ( $\text{Fe}^{2+}/\text{H}_2\text{O}_2/\text{h}\nu$ /natural water). Consequently, it can be assumed that iron as the catalyst of the photo-Fenton process is not necessary in high concentrations to inactivate enteric micro-organisms. The lower efficiency of the system  $\text{Fe}^{2+}/\text{H}_2\text{O}_2/\text{h}\nu$ /natural water may also indicate that an optimum concentration of iron for an efficient photo-Fenton process exists. Indeed, at near neutral pH (6.3), a part of added iron salts precipitate and negatively affects the color and turbidity and light transmittance and leads to a concomitant decrease in the disinfection efficiency. This result suggests the use of Sahelian Fe-containing region to perform a photo-Fenton treatment of drinking water by adding  $\text{H}_2\text{O}_2$  only.

The fastest inactivation kinetic of *Salmonella* spp. compared to that of the total coliforms/*E. coli* in the water with the near-neutral pH (W2, pH: 6.3) brings us to the assumption that the pH can significantly influence the resistance of these enteric bacteria to photo-catalytic inactivation. The results have to be confirmed by further research, however in order to establish the best disinfection process considering all the parameters affecting wild enteric bacteria inactivation.

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